# Exhibit A

## 1. Materials and Methods

### 1.1. *Human Peripheral Blood Leukocytes*

Leukaphoresis samples from healthy normal donors expressing HLA-A2 were obtained from SeraCare LifeSciences (Oceanview, CA). Peripheral Blood Leukocytes (PBL) were purified on Ficoll (Histopaque 1.077, Sigma, St. Louis, MO), aliquoted and frozen.

### 1.2. *Antibodies*

MAb-B43.13 and AR9.6 are murine monoclonal IgG1 antibodies to CA125 (Unither Pharmaceuticals.).

### 1.3. *Antigens*

CA125 was purified from tissue culture supernatant of NIH:OVCAR-3 cells purchased from ATCC (Manassas, VA).[i] The purification steps included ammonium sulfate cut, diafiltration, gel filtration and an anti-CA125 antibody affinity column.

### 1.4. *Monocyte, Dendritic Cell (DC) and T Cell Preparation*

Human monocytes were prepared from PBL's after negative selection with anti-CD2, CD7, CD16, CD19, and CD56 antibodies, followed by anti-mouse-Ig-magnetic beads (Dynal-Invitrogen Monocyte Negative Selection Kit). These negatively selected cells were 70-85% pure monocytes as characterized using a broad CD marker panel and forward and side scatter by flow cytometry (BD FACSCalibur™ flow cytometer and BD CellQuest™ software). To generate immature DCs, monocytes were cultured in GM-CSF and IL-4 (1000 U/mL each, R&D Systems, Minneapolis, MN) for 6 days in RPMI + 2% human AB serum.

To induce DC maturation, Tumor Necrosis Factor (TNF)-α (10 ng/mL, Biosource) and Interferon (IFN)-α (50 U/mL, PBL Biomedical Laboratories) were added to immature DCs on Day 6 of culture. Mature DCs were characterized using a broad CD marker panel and gating based on forward and side scatter by flow cytometry on Day 7.

T cells were prepared from PBL by negative selection with anti-CD14, CD16, CD56, and HLA-DR/DP, followed by anti-mouse-Ig-magnetic beads (Dynal T Cell Negative Selection Kit). These negatively selected cells were approximately 80-90% pure T cells as characterized using a T cell and lineage CD marker panel as well as gating based on forward and side scatter by flow cytometry. T cells were used immediately following isolation.

### 1.5. *T Cell Activation Assays with DC*

Immature DCs were loaded with antigen, antibody or antigen-antibody complexes at Day 6 and matured with TNF-α and IFN-α (10 ng/mL and 50 U/mL, respectively) 2-4

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h later. On Day 7, DC were washed and purified T cells were added at a ratio of 10:1 (T cells to DC). Two or three stimulation rounds were performed. Before analyzing the T cells for activation, T cells were re-stimulated with antigen loaded DC for 24 h at a T cell:DC ratio of 20:1.

### *1.6. Quantitation of T Cell Activation by Intracellular Cytokine (ICC) Staining for IFN-γ*

Cells were incubated with Golgi-Plug (R&D Systems) 2 h after stimulation with the antigen-loaded DC, incubated for another 16-20 h, blocked and stained with anti-CD3-FITC and anti-CD8-Cy-Chrome (all from Pharmingen) for 30 min on ice. Cells were washed, permeabilized with Perm/Fix (Pharmingen) for 30 min on ice, washed and stained with anti-IFN-γ-PE antibody (Pharmingen) for 30 min on ice. Cells were washed, fixed in 0.5% formalin and analyzed by flow cytometry (FACS Calibur, Becton Dickinson), gating on CD3-positive lymphocytes.

### *1.7. Cytotoxic T Lymphocyte Assay (CTL)*

T cells were harvested 24 h after stimulation with loaded DC and used to set-up the CTL assay. As targets cells, CA125-positive HLA-A2 expressing ovarian cancer cells (NIH:OVCAR-3, ATCC) were used. The target tumor cells (1-2x$10^6$ cells) were labeled with Calcein-AM (Molecular Probes) according to the manufacturer's instructions. The cells were incubated for 30 min. at 37°C with occasional mixing followed by washing the cells in 3-times with 10 mL of medium. The cells were resuspended at 1x$10^5$ cells/mL, and 100 µL was distributed into round-bottom microtiter plates.

T cells from each group were washed and resuspended at 1, 0.3 and 0.1 x$10^6$ cells/mL, and 100 µL/well for each dilution were plated to the labeled target cells in triplicates, including controls with target cells alone (spontaneous release) and target cells lysed with 0.1% Triton X-100 (maximum release). The plates were incubated for 4 h at 37°C after a 3 min centrifugation at 30 x *g*. The Calcein-AM release (tumor killing) into the supernatant was measured with a fluorescence plate reader.

### *1.8. Analysis of ICC and CTL*

For the ICC experiments, the % positive cell data were acquired on the flow cytometer and gates set based on isotype controls. For the CTL assay the specific lysis was calculated based on the following formula:

$$\% \text{ lysis} = \frac{\text{mean released test results} - \text{mean spontaneous released results}}{\text{mean maximum released results} - \text{mean spontaneous released results}}$$

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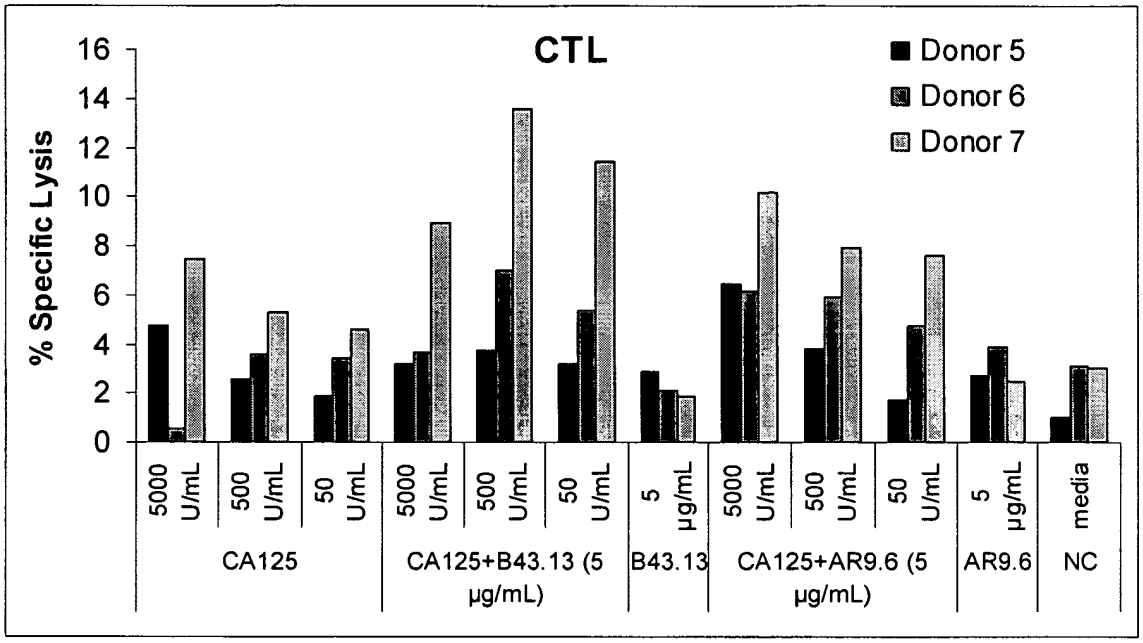
# 2. Results

## 2.1. *CTL Assay after 3 Rounds of Stimulation*

T cells were analyzed 24 h after the 3rd stimulation with loaded DC for lysis of CA125-positive tumor cells using three different donors. Lysis for T cells stimulated with CA125 alone (50-5000 U/mL) or with antibody alone (5 μg/mL) ranged form 2-7.5%. Immune complexes of CA125 and B43.13 or AR9.6 at the same concentrations stimulated T cells more effectively, resulting in lysis of up to 14% of the tumor cells.

**GRAPH 1**




Immature DC from 3 donors were loaded with CA125 alone (50, 500, and 5,000 U/mL), MAb-B43.13 or AR9.6 (5 μg/mL), CA125 + MAb-B43.13 (50, 500, or 5,000 U/mL + 5 μg/mL), or CA125 + AR9.6 (50, 500, or 5,000 U/mL + 5 μg/mL). DC were matured for 24 hrs and then washed 2 times with media. Purified T cells from the matching donor were added and incubated for 7 days. T cells were re-stimulated with DC loaded as before for an additional 2 rounds. Twenty-four hours after the final stimulation, T cells were harvested and analyzed for lysis of NIH:OVCAR 3 cells in a Calcein-AM release assay.

## 2.2. *CTL Assay after 4 Rounds of Stimulation*

T cells were analyzed 24 hrs after the 4th stimulation with loaded DC for lysis of CA125-positive tumor cells using three different donors. Lysis for T cells stimulated with CA125 alone or with antibody alone ranged form 0-17%. Processing of immune complexes of CA125 and B43.13 or AR9.6 by autogous DC resulted in significantly higher CTL activation. Tumor cell lysis ranged from 12-41%.

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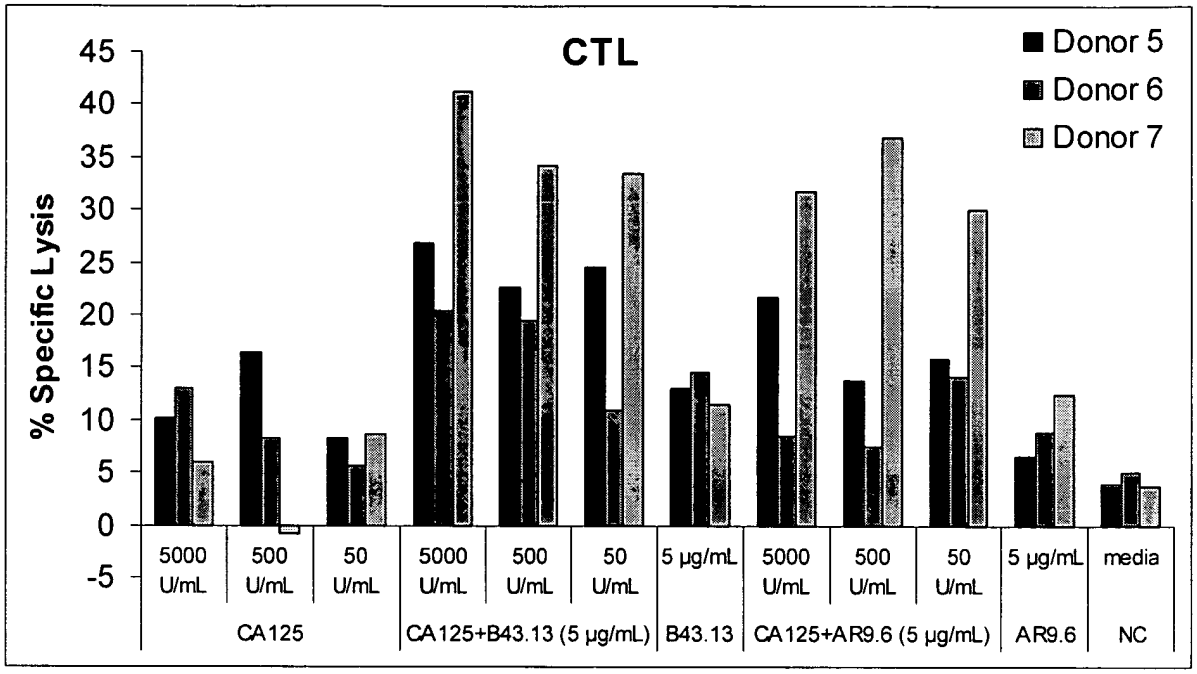
**GRAPH 2**




Immature DC from 3 donors were loaded with CA125 alone (50, 500, and 5,000 U/mL), MAb-B43.13 or AR9.6 (5 µg/mL), CA125 + MAb-B43.13 (50, 500, or 5,000 U/mL + 5 µg/mL), or CA125 + AR9.6 (50, 500, or 5,000 U/mL + 5 µg/mL). DC were matured for 24 hrs and then washed 2 times with media. Purified T cells from the matching donor were added and incubated for 7 days. T cells were re-stimulated with DC loaded as before for an additional 3 rounds. Twenty-four hours after the final stimulation, T cells were harvested and analyzed for lysis of NIH:OVCAR 3 cells in a Calcein-AM release assay.

## 2.3. *Intracellular IFN-γ Staining after 4 Rounds of Stimulation*

T cells were stimulated with DC loaded with CA125 alone, B43.13 alone, AR9.6 alone, or immune complexes of CA125+B43.13 or CA125+AR9.6 for 4 rounds. Two hours after the final stimulation, wells were treated with Brefeldin A to block cytokine secretion and incubated overnight. The following day, T cells were harvested and washed, stained for surface CD3 and CD8, fixed, permeabilized and stained for intracellular IFN-γ. Cells were analyzed by flow cytometry. Results from 3 different donors are shown. T cell activation for cells stimulated with DC processing CA125 alone or antibody alone was significantly lower than for cells stimulated with DC that processed immune complexes of CA125+B43.13 or CA125+AR9.6.

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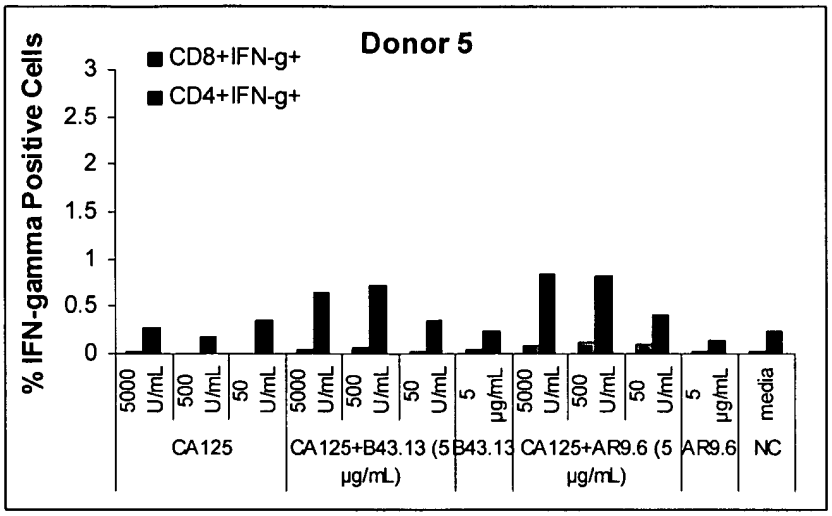
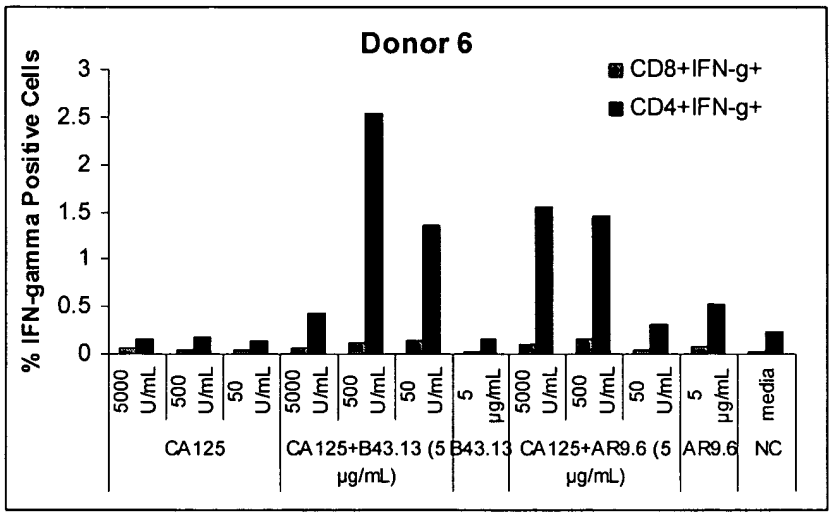
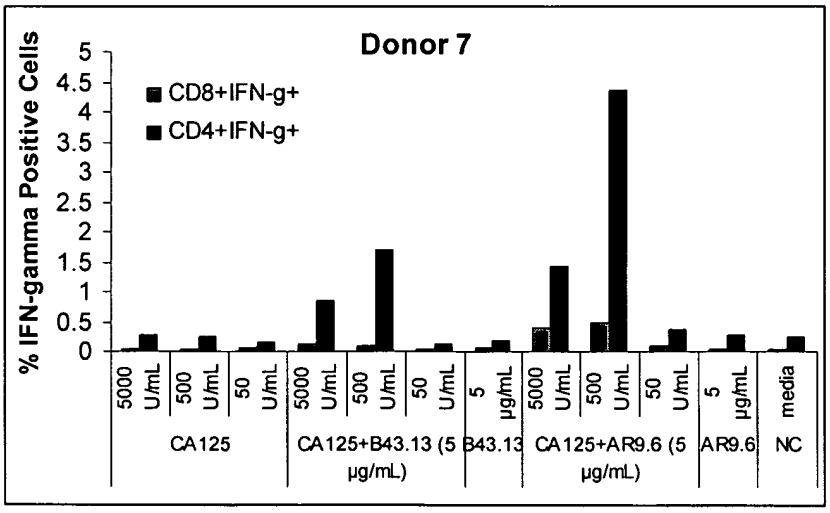



GRAPH 3




GRAPH 4




GRAPH 5

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Immature DC from 3 donors were loaded with CA125 alone (50, 500, and 5,000 U/mL), MAb-B43.13 or AR9.6 (5 μg/mL), CA125 + MAb-B43.13 (50, 500, or 5,000 U/mL + 5 μg/mL), or CA125 + AR9.6 (50, 500, or 5,000 U/mL + 5 μg/mL). DC were matured for 24 hrs, and then washed 2 times with media. Purified T cells from the matching donor were added and incubated for 7 days. T cells were re-stimulated with DC loaded as before for an additional 3 rounds. Wells were treated with Brefeldin A and 24 hrs after the final stimulation, T cells were harvested, stained for CD3, CD8 and IFN-γ and analyzed by flow cytometry.

## 3. References

[i] Schultes BC, Baum RP, Niesen A, Noujaim AA, Madiyalakan R. Anti-idiotype induction therapy: anti-CA125 antibodies ($Ab_3$) mediated tumor killing in patients treated with Ovarex mAb ($Ab_1$). Cancer Immunol and Immunther (1998) 46:201-212.